Renal dysplasia in grey Alpine breed cattle unrelated to CLDN16 mutations

S. Testoni, S. Mazzariol, C. Drögemüller, C. Piffer, L. Aresu, A. Gentile

Renal dysplasia (RD) is a developmental disorder of the renal parenchyma characterised by anomalous differentiation of the nephrons and collecting ducts (Woolf and others 2004). In human medicine, RD is one of the principal causes of childhood end-stage renal failure. In veterinary medicine, it has been described in several species, including cattle (Dunham and others 1989, Simon and others 1999, Ohba and others 2001, Castro and others 2007, Maxie and Newman 2007, Aresu and others 2009, Philbey and others 2009). In Japanese Black cattle, RD shows autosomal recessive inheritance, caused by CLDN16 mutations (Hirano and others 2000, Ohba and others 2000, Hirano and others 2002). These mutations affect one of the members of the claudin family of genes which plays an important role in the formation of tight junctions in the kidney (Simon and others 1999). In Japanese Black cattle, RD is classified into two types, according to two independent CLDN16 mutations (Hirano and others 2002), but no morphological or histopathological differences between the two types have been reported. This short communication deals with a case of RD in twin grey Alpine heifers which are not related to mutations of the CLDN16 gene.

Eight-month-old twin grey Alpine heifers, (cases 1 and 2) were visited for growth retardation and overgrowth of hooves beginning at the age of two to three months. Despite normal or only slightly decreased appetite and intense vitamin integration, the animals had gradually lost weight and showed impaired skeletal development. Their hooves had needed to be trimmed repeatedly.

Pedigree analysis of the parents revealed two common male ancestors at the level of the third and fourth generation, respectively.

At clinical examination, both animals were depressed and sluggish. In particular, case 1 was reluctant to stand up and needed passive help to be put into a quadrupedal stance. Both were stunted and emaciated; their bodyweight was 75 (patient 1) and 80 (case 2) kg (expected weight around 200 kg). All the hooves were overgrown and misshapen (Fig 1). Matted and coarse coats, dehydration, pale mucous membranes and poor appetite were also observed.

Haematological parameters were within the reference range. On the contrary, clinical biochemistry indicated the presence of renal failure (Table 1). Blood samples tested for bovine viral diarrhea virus (BVDV) antigens and for antibodies to BVDV, bovine Herpes virus 1 and Neospora caninum gave negative results.

Ultrasonographic examination of the right kidney and urinary bladder was performed in case 2 on day 2 after admission. The renal cortex of the right kidney was hyperechoic when compared with the liver and hyperechoic when compared with the renal medulla; the latter was clearly hyperechoic and cast acoustic shadows via the deeper tissues. There was decreased visualisation of the internal renal architecture. In the sinus, the renal calices were dilated with anechoic fluid and showed multiple small hyperechoic half-moon-shaped calculi.

FIG 1: Calf (case 2) affected by renal dysplasia. Note the matted and coarse coat, and the overgrown and misshapen hooves

FIG 2: Transverse sonogram of the right kidney (case 2). Note the hyperechoic renal cortex (c), and the even more hyperechoic renal medulla (m), the latter casting acoustic shadow (s)
 casting acoustic shadows (Fig 2). The urinary bladder showed hyper-echoic content in the ventral portion of the bladder casting an acoustic shadow. The material was easier to see upon ballottement; it was free-floating in the lumen and suspended in a fluid medium. The bladder wall was echogenic, irregular and thick, and appeared as two thin hyperechoic lines separated by a thick hypoechoic band in the middle. The wall thickness was 5 mm.

Case 1 worsened rapidly; on day one after admission, it was hypo-thermic (rectal temperature 35°C) and died. Case 2 had a more gradual worsening of its general condition. Severe dullness, inappetence, dehydration and recumbency prompted euthanisation at day 15 after admission.

On gross postmortem examination, the findings were similar in both animals. The kidneys were hypotrophic, firm and pale, and had a roughened and granular surface. The capsule was slightly adherent to the irregular subcapsular surface. At the cut surface, dense cortical and medullar fibrosis, and fibrous wedges extending from the pelvis to the cortex were evident. Mild dilation of the pelvis with free yellow calculi and disseminated medullar mineralisation were also observed.

Small stones (0.3 to 0.5 cm in diameter) were also found in the ureters and in the bladder.

At histopathology (Periodic Acid Schiff, Acid Fuchsia Orange G, Periodic Acid Silver Methanamine and Masson’s trichrome staining), the kidneys of both animals showed diffuse and massive infiltration of immature mesenchymal tissue forming a net with evident parallel ridges, connected with transversal bridges, which gave a pseudo-lobular appearance to the renal tissue (Fig 5). The cortex was thin and presented primitive tubules lined by cuboidal epithelium on a thickened basal membrane, and immature glomeruli at different developmental stages (Fig 4). Cystic dilation of Bowman’s capsule with marked periglomerular fibrosis and sclerotic glomeruli were also present. Scattered interstitial infiltrates of lymphocytes and plasma cells were found in the interstitium associated with very marked dilation of the tubules. The medulla showed persistent mesenchyme and many primitive tubules lined by pseudostratified, columnar, epithelial cells. There were many dilated collecting ducts lined by flattened epithelial cells with scant cytoplasm and hyperchromatic nuclei. Adenomatoid proliferation of the cuboidal epithelial cells was frequently observed. On the basis of all the aforementioned findings, a diagnosis of RD was made.

Genomic DNA was therefore extracted from EDTA stabilised blood samples of the affected calves and their dam as well as from a single unrelated control cow of the grey Alpine breed. DNA was screened for two different mutations of the \( CLDN16 \) gene responsible for inherited renal tubular dysplasia in Japanese Black (Wagyu) cattle (Ohba and others 2000, Hirano and others 2002). Therefore, all five exons and the parts of the flanking introns, including the conserved splice sites of the bovine \( CLDN16 \) gene, were PCR amplified and subsequently sequenced. After successful amplification of all five \( CLDN16 \) exons in the four individuals, the presence of a genomic deletion, such as that in Wagyu cattle, was excluded. Finally, the re-sequencing of the PCR products revealed no polymorphisms affecting the coding sequence or consensus splice sites of \( CLDN16 \).

Both clinically and histopathologically, the RD seen in the grey Alpine heifers resembled what has been described in RD of Japanese Black cattle: dullness, growth retardation, overgrowth of hooves and severe renal failure on a clinical basis (Ohba and others 2001), and inma-

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**TABLE 1: Clinical biochemistry in two eight-month-old twin calves affected by renal dysplasia**

<table>
<thead>
<tr>
<th>Cases</th>
<th>Blood urea nitrogen (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Phosphorus (mg/dl)</th>
<th>Calcium (mg/dl)</th>
<th>Total protein (g/l)</th>
<th>Glucose (mg/dl)</th>
<th>CK (UI/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>743</td>
<td>16.3</td>
<td>2.1</td>
<td>1.8</td>
<td>33</td>
<td>256</td>
<td>267</td>
</tr>
<tr>
<td>2†</td>
<td>298</td>
<td>6.3</td>
<td>12.1</td>
<td>8.5</td>
<td>78</td>
<td>68</td>
<td>905</td>
</tr>
<tr>
<td>2§</td>
<td>473</td>
<td>7.1</td>
<td>19.7</td>
<td>6.7</td>
<td>55</td>
<td>74</td>
<td>1095</td>
</tr>
<tr>
<td>Normal</td>
<td>43-64</td>
<td>1-2</td>
<td>5.6-6.5</td>
<td>9.7-12.0</td>
<td>67-75</td>
<td>45-75</td>
<td>50-160</td>
</tr>
</tbody>
</table>

* Day 1 after admission  
† Day 1 after admission  
§ Day 13 after admission  
CK Creatine kinase
ture tubules and glomeruli, reduction in the number of glomeruli, hypertrophy of the glomeruli, glomerular and tubular atrophy accompanied by interstitial fibrosis and lymphocytic infiltration and cystic dilation of the tubules on a histopathological basis (Sasaki and others 2002, Woof and others 2004). However, despite the strong similarities, no evidence of the existence of causative CLDN16 mutations was found.

Interestingly, the literature quotes other cases of RD in calves which were not related to mutations of the CLDN16 gene (Dunham and others 1989, Castro and others 2007, Sugiyama and others 2007, Philbey and others 2009) but none of them showed the complete overlapping observed in this study, especially referring to the overgrowth of the hooves.

Congenital RD has also been reported in association with BVDV infection acting as a teratogenic agent (Maxie and Newman 2007). The negative results, however, excluded this possibility in the grey Alpine heifers in this study.

In conclusion, this study showed a nephrologic syndrome phenotypically overlapping RD in Japanese Black cattle but which is not associated with mutations affecting the CLDN16 gene. The results confirm that RD should be considered etiologically heterogeneous, as it is in human medicine (Sanna-Cherchi and others 2007). A larger number of cases are necessary to make an in-depth study of this disease and to better clarify whether it might be considered of genetic origin or if other causes (such as infective or toxic agents) might be responsible for the defect.

Hopefully, this paper will alert bovine practitioners and breeders the importance of reporting animals with the above-mentioned clinical findings to research or diagnostic centers for thorough evaluation.

References


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